INTRODUCTION :

Cotton is of the genus Gossypium, of the tribe Gossypieae, and of the family Malvaceae. Worldwide, four species of cotton are of agronomic importance: the two diploid Asiatic species, *G. arboreum* and *G. herbaceum,* and the two-allotetraploid New World species, G. *barbadense* and G. *hirsutum.* Wild populations of *G. hirsutum* are relatively rare and tend to be widely dispersed. All grow on beach strands or on small islands. Cotton is normally considered a self-pollinating crop but can be cross-pollinated by certain insects. However, outcrossing of the cryAb2 gene from Bollgard II cotton to other Gossypium species or to other Malvaceous genera is extremely unlikely for the following reasons (Percival et al., 1999).

In India, cotton is primarily grown in dry tropical and sub tropical climates at mean temperatures between 11°C and 25ºC with a rainfall of 250-1500 mm. It is cultivated in India from sub Himalayan region of Punjab in the North to TamilNadu in South and dry regions in the East.

Cotton (*Gossypium* sp.) is an important fiber crop of India covering an area of 88.20 lakh hectares with production of 242.5 lakh bales and productivity of 467 kg lint per hectare. In Karnataka, it is grown on an area 3.8 lakh hectares producing 7.0 lakh bales with productivity of 312 kg lint per hectare.

The most commonly cultivated species of cotton in the world include *Gossypium hirsutum.*

Thus, one can define a GM food/crop/organism as a functional foreign genes, incorporated by genetic engineering that are not present naturally in them, are termed as **Transgenic organisms or Genetically modified organisms (GMOs)** and foreign gene insert is called a **Transgene.**

The **first genetically engineered products eaten by humans** were **cheese and tomatoes**. Stanley N. Cohen and Herbert Boyer demonstrated the creation of first functional GMO in 1973 that combined and replicated genetic information from different species. GM bacteria were first used to produce an alternative enzyme, **chymosin**, to calf rennet so as to make vegetarian cheese. The **first transgenic animals** were mice created by Rudolf Jaenisch at Ohio University in 1974. Jaenisch successfully inserted foreign DNA into the early-stage

mouse embryos; the resulting mice carried the modified gene in all their tissues. The **first transgenic plant** was produced in **1983**, when a tobacco line expressing kanamycin resistance was produced. **'Flavr Savr' tomato** was **the first transgenic variety** to reach the **market;** fruits of this variety remain fresh for a prolonged period as their softening gene was **'switched-off ',** allowing them to ripen on the vine until they reach full flavor and color without rotting.

But the greatest growth has been seen in crops containing one of two traits:

- **Herbicide tolerance**, mainly in **soybean, oil seed rape (canola) and sugar beet**, which allows the application of broad-spectrum herbicides to the standing crop, without causing damage but killing all the weeds.
- **Insect resistance through *Bacillus thuringiensis* (Bt) expression, mainly in maize and cotton**, which means that the Bt insecticidal toxin is expressed by all cells of the plant, so killing herbivorous pests and theoretically reducing the need to apply conventional pesticides.

In India, genetically modified foods/organisms are regulated under the purview of the 1986 Indian Environment (Protection) Act (henceforth, the EP Act). These rules and regulations cover the areas of research as well as large-scale applications of GMOs and products, throughout the country involving manufacture, use, import, export, storage and research. The target substances covered are all genetically engineered organisms including microorganisms, plants and animals. The Government is following a policy of case-by-case approval of transgenic crops. Bt. Cotton is the first and only transgenic crop approved by GEAC (regulatory part of DBT) for commercial cultivation in 6 States of India namely Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh, Maharashtra and Tamil Nadu.

A common example of genetic engineering is the insertion of *Bacillus thuringiensis* genes into corn to make Bt- cotton *Bacillus thuringiensis* is a bacterium that naturally produces a protein that is lethal to insect larvae. By transferring the genes that encode this protein into cotton balls, scientists have created a type of cotton that produces its own pesticides, making it resistant to insects such as the Bt-cotton event Bollgard II.

Many countries have introduced legislation regulating the approval release of GMOs. In US three independent authorities are involved in regulation of release of genetically engineered plants & their use a food stuffs: APHIS (Animal &Plant Health Inspection Service), FDA (Food & Drug Administration), EPA (Environmental Protection Agency). Several countries have approved GMOs viz the U.S., Canada, European Union , Switzerland, Australia, Argentina, Brazil& Japan.

Cotton (*Gossypium hirsutum* L.) is not a crop mainly consumed by humans as food, it is an important economic crop in the world because its boll can be used as fiber, its seed oil as a cooking oil or snack food, and its meal and hulls as important protein concentrates for livestock (Lin.xi Jiang etal 2009). TheMON15985 event was produced based on a previously commercialized Lepidopteran resistant cotton variety known as MON531, expressing a chimeric form of a Cry1A insect resistant protein. In the MON15985 event, one copy of complete t-DNA containing the *Cry2Ab* gene was integrated at a single site in host cotton genomic DNA

Bt-Cotton (BollgardII) Event 15985

Bollgard II cotton event 15985 was developed by Monsanto Company to produce the Cry2Ab insect control protein, which provides effective season-long control of key lepidopteran insect pests. This product was produced by re-transformation of Bollgard® cotton event 531, which produces the Cry1Ac insect-control protein and the NPTII selectable marker protein. Therefore, Bollgard II cotton produces two proteins for effective control of the major lepidopteran insect pests of cotton, including the cotton bollworm, tobacco budworm, pink bollworm, and armyworm. Bollgard II cotton also produces the ß-D-glucuronidase (GUS) marker protein. In addition, Bollgard II cotton provides a more effective insect resistance management program compared to single gene products.

Bollgard cotton has been grown globally on more than 32 million acres since commercial introduction in 1996 (James, 2002). The primary benefits that have resulted from the use of Bollgard cotton are reduced insecticide use, improved control of target insect pests, improved yield, reduced production costs, and improved profitability for cotton growers .

The development and commercialisation of GM crops is increasing at a faster pace, to develop qualitative and quantitative methods for detection of GM crops has become even more challenging. In India, till date, six events of Bt cotton, i.e. MON531 with cry1Ac gene,

MON15985 with cry1Ac and cry2Ab genes, GFM with fused cry1Ac-cry1Ab gene, Event 1 with cry1Ac, Event 9124 with synthetic cry1C gene and Dharwad event (Bikaneri Nerma-Bt variety) with truncated cry1Ac gene have been commercialised, which are being cultivated in an area of 8.4 million ha (James ,Karihaloo,Kumar,2009).

Polymerase chain reaction (PCR) is the most widely used and accepted analytical method for GM detection. Multiplex PCR, a derivative of conventional PCR, is reliable, efficient and cost-effective qualitative assay, as fewer reactions are required to test the transgenic nature of a crop by simultaneously detecting the target sequences of the inserted gene construct, i.e. specific transgene, marker genes, promoter and terminator gene sequences in a single PCR assay (Randhawa etal 2009a).

Objectives :-

- To identify & selection of Transgenic plant available.
- To recognize the transgenes present in the available transgenic plant.
- To design primers& probes ourselves or using already published validated ones.
- To Detect & Quantitate transgenes & housekeeping genes using PCR & Real Time PCR techniques.
- To explore other modern tools of detection.

MATERIALS AND METHODS:

Collection of samples

The Bt- cotton and its counterpart was collected from Main Cotton Research Station Navasari Agricultural University, Athwa Farm,Ghod dhod Road Surat was only meant for research purposes. A complete list of Bt-cotton and its counterpart was given below& store for –20°C for long-term storage.

1-Gujarat Cotton Hybrid-6(Non Bt)
2-Gujarat Cotton Hybrid-6(BG-II) Bt
3-Gujarat Cotton Hybrid-8(Non Bt)
4-Gujarat Cotton Hybrid-8(BG-II) Bt
5-Guajrat Cotton 10(Non Bt)
6-Gujarat Cotton 10 (BG-II) (Bt).

1.Isolation of Plant Genomic DNA

2.Spectrophotometric quantitation of DNA

3.Polymerase Chain Reaction

4.ELISA TEST-

5.Cloning of Bt-Cotton transgene of Cry1A(c) and Cry2Ab

6.Sequencing

RESULTS:

Expected deliverables/outcome

- Confirmation of Housekeeping gene in GM as well as control variety.
- Detection of target gene i.e. transgene is selected in Transgenic plant.
- Multiplex detection of Transgene, Housekeeping gene promoter and terminator gene.

Significance of the expected outcome with respect to the state-of-the-art-in the field-

Till date not a single GM food crop is being cultivated in India as there is a lot of public debate going on however globally there are many GM crops in the market and they are being imported in the country whereas Indian Government still debating for the implementation of the threshold limits as already approved by other countries in the world.

So keeping this in view and to check the unauthorized import of transgenic material this attempt will be helpful in early detection of transgenes.

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